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# *Triticum dicoccoides*: An Important Genetic Resource for Increasing Zinc and Iron Concentration in Modern Cultivated Wheat

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**One major strategy to increase the level of zinc (Zn) and iron (Fe) in cereal crops, is to exploit the natural genetic variation in seed concentration of these micronutrients. Genotypic variation for Zn and Fe concentration in seeds among cultivated wheat cultivars is relatively narrow and limits the options to breed wheat genotypes with high concentration and bioavailability of Zn and Fe in seed. Alternatively, wild wheat might be an important genetic resource for enhancing micronutrient concentrations in seeds of cultivated wheat. Wild wheat is widespread in diverse environments in Turkey and other parts of the Fertile Crescent (e.g., Iran, Iraq, Lebanon, Syria, Israel, and Jordan). A large number of accessions of wild wheat and of its wild relatives were collected from the Fertile Crescent and screened for Fe and Zn concentrations as well as other mineral nutrients. Among wild wheat, the collections of wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides* (825 accessions) showed impressive variation and the highest concentrations of micronutrients, significantly exceeding those of cultivated wheat. The concentrations of Zn and Fe among the *dicoccoides* accessions varied from 14 to 190 mg kg$^{-1}$ DW for Zn and from 15 to 109 mg kg$^{-1}$ DW for Fe. Also for total amount of Zn and Fe per seed, *dicoccoides* accessions contained very high amount of Zn (up to 7 μg per seed) and Fe (up to 3.7 μg per seed). Such high genotypic variation could not be found for phosphorus, magnesium, and sulfur. In the case of modern cultivated wheat, seed concentrations of Zn and Fe were lower and less variable when compared to wild wheat accessions. There was a highly significant positive correlation between seed concentrations of Fe and Zn. Screening different series of *dicoccoides* substitution lines revealed that the chromosome 6A, 6B, and 5B of *dicoccoides* resulted in greater increase in Zn and Fe concentration when compared to their recipient parent and to other chromosome substitution lines. The results indicate that *Triticum turgidum* L. var. *dicoccoides* (wild emmer) is an important genetic resource for increasing concentration and content of Zn and Fe in modern cultivated wheat.**

***Key Words:*** **grain quality, iron, wheat, zinc.**

The world population is expanding rapidly with increased number of micronutrient deficient people, particularly in developing countries. Recent estimates indicate that over 3 billion people are afflicted by Fe deficiency (Welch and Graham 1999, 2004), and up to one-third of the population in developing countries are at risk of Zn deficiency (Hotz and Brown 2004). Iron and Zn deficiencies cause severe health complications including impairments in the immune system, physical growth, mental and cognitive development, and increases in anemia, morbidity, and mortality (Black 2003; Boccio and Iyenger 2003; Hotz and Brown 2004). Micronutrient deficiencies are also associated with reduced work productivity and involved in decrease in gross national product in developing countries, such as in Bangladesh (Bouis 2003).

Iron and Zn deficiencies are of increasing concern in developed countries as well. Iron deficiency anemia, defined as a hemoglobin level less than 11 g dl$^{-1}$, is widespread in children in the United Kingdom, where

between 11 and 38% of children under two years of age are reported to suffer from this condition (James and Laing 1994). An estimated 10% of the population in the USA and Canada also are at risk of Zn deficiency (Hotz and Brown 2004). In Turkey, according to the Micronutrient Initiative reports (www.micronutrient.org/IDPAS), 50% of the 6-months old children, 30% of school age children and 50% of women of reproductive age have Fe deficiency anemia.

A major reason for widespread occurrence of Fe and Zn deficiencies in developing countries is the high consumption of diets with little diversity, often consisting of mainly one or two staple foods. Due to widespread poverty, the majority of people in the developing world rely on cereal-based foods as a source of energy and protein intake, and the animal-based food products with high levels of micronutrients are very rarely consumed. Cereal-based foods contain low levels of Zn and Fe, and most of the Zn and Fe present in seeds are lost during milling or polishing (Cakmak et al. 2002; Poletti et al. 2004; Welch and Graham 2004). In less developed countries, wheat, maize, and rice, depending on the region are the predominant stable food in the diet. Wheat provides up to 60% of the daily calorie intake (Fig. 1). Any increase in Zn and Fe concentrations in wheat seeds will, therefore, have a significant impact on reducing micronutrient deficiencies. Cereal do not only contain relatively low levels of Zn and Fe, but are also rich in compounds limiting bioavailability of Zn and Fe in the body, such as phytate and fibre (Frossard et al. 2000; Cakmak et al. 2002; Welch and Graham 2004).

Food fortification and supplementation with micronutrients has been discussed to address micronutrient deficiencies, but this strategy is considered too expensive and not practical to be applied on large scale in developing countries, particularly in rural regions (Bouis et al. 2000; Bouis 2003). Alternatively, enrichment of cereals with Zn and Fe and the improvement of their bioavailability in seeds through traditional plant breeding methods or genetic engineering is the most cost-effective and sustainable solution to Fe and Zn deficiencies (Frossard et al. 2000; Cakmak et al. 2002; Poletti et al. 2004; Welch and Graham 2004).

Increasing micronutrient concentration of cereal seeds through breeding requires the existence of substantial and useful genetic variation for micronutrients in seeds. Several authors have reported the genotypic variation for Zn and Fe in cereal seeds (Peterson et al. 1986; Graham et al. 1999; Rengel et al. 1999; Cakmak 2002). The range of the observed genetic variation for Zn and Fe within cultivated wheat varieties and advanced breeding lines is, however, relatively small, and most likely cannot contribute to development of genotypes with considerably higher levels of Zn and Fe. Furthermore, genotype × environment (G × E) interaction is very high and factors such as soil properties, water availability and fertilizer management have much greater effect on the micronutrient concentration than the genetic factors (Peterson et al. 1986; Bänziger and Long 2000). In preliminary studies, wild and primitive wheat, such as *Triticum monococum*, *Triticum dicoccon*, and *Triticum dicoccoides* were found to be promising genetic donors for micronutrients, more than cultivated wheat cultivars and advanced breeding lines (Cakmak et al. 1999a, 2000; Ortiz-Monasterio and Graham 2000). Among wild wheat germplasm, the emmer wheat, *Triticum dicoccoides*, showed the largest variation and the highest concentration of micronutrients, especially for Zn, and is considered to be one of the most promising donors to improve Zn and Fe concentrations of wheat seeds (Cakmak et al. 2000). Higher levels of Zn in seeds also contribute to better growth and yield of plants under Zn-deficient conditions as shown in pot (Rengel and Graham 1995) and field experiments (Yilmaz et al. 1998).

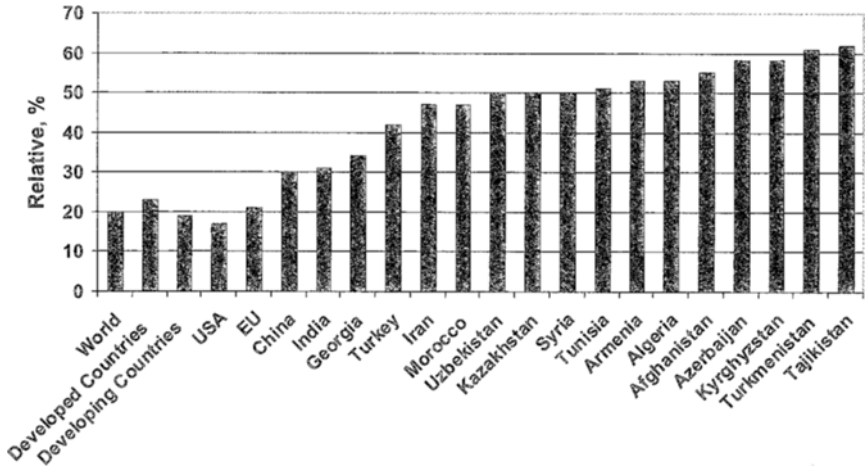


**Fig. 1.** Daily calories intake from wheat in different countries and regions (source: FAO Database 2003; compiled by H.J. Braun, CIMMYT-Turkey).

**Table 1.** Seed concentration of Fe and Zn of various *Triticum turgidum* ssp. *dicoccoides* germplasms from different sources.

| Germplasms[a] | Number of genotypes | Concentration (mg kg$^{-1}$ dry wt.) | | | | Content (μg seed$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Fe | | Zn | | Fe | | Zn | |
| | | Median | Range | Median | Range | Median | Range | Median | Range |
| I—Field | 113 | 40 | 24–96 | 63 | 35–100 | 1.13 | 0.44–2.44 | 1.83 | 0.74–3.45 |
| II—Field | 518 | 40 | 15–94 | 61 | 30–98 | 1.23 | 0.30–2.30 | 1.89 | 0.54–3.67 |
| III—Field | 83 | 57 | 26–109 | 60 | 32–97 | 1.04 | 0.32–2.44 | 1.10 | 0.37–2.65 |
| IV—Greenhouse | 111 | 48 | 21–91 | 88 | 14–190 | 1.28 | 0.32–3.72 | 2.36 | 0.26–6.81 |

Each value represents the mean ± SD of two replications. [a]Germplasm-I was obtained from the Haifa University, germplasm-II from the Tel Aviv University, germplasm-III from the Cukurova University, and germplasm-IV from the Weizman Institute of Science. Germplasms-I, II, and III were grown in field and germplasm-IV was planted in greenhouse.

In this manuscript we presented data on the natural variation for Zn, Fe, Mg, P, and S in seeds of 825 *Triticum dicoccoides* accessions collected from sites in the Fertile Crescent region. In addition, the chromosomal localization of genes affecting high levels of Zn and Fe in seeds has been studied by using 2 different sets of *dicoccoides* substitution lines, e.g., *Triticum aestivum* cv. Chinese Spring (CS)–*Triticum dicoccoides* and Langdon (LNG)– *Triticum dicoccoides*.

## MATERIALS AND METHODS

In the present study, seeds of 825 wild emmer accessions (*Triticum turgidum* ssp. *dicoccoides*) have been used for analysis of Zn, Fe, P, Mg, and S. The accessions have been collected from different sites in the Fertile Crescent region. As presented in Table 1, four different germplasms have been used. The germplasm-I was received from the Gene Bank of the Institute of Evolution, Haifa University. This germplasm has been collected on different places in Iran, Turkey, and Israel, and then they were grown under same year and field conditions in Haifa (in the location Atlit). The seeds of the germplasm-II have been received from the Gene bank of the Institute for Cereal Crops Improvement, Tel Aviv University. All accessions of this germplasm have been collected from different places in Israel, and then they were grown under same year and field conditions in the research farm of the Institute for Cereal Crops Improvement, Tel Aviv University. The germplasm-III has been collected from Turkey, Syria, Lebanon, Israel, and Jordan, and grown under same year and field conditions in the research farm of the Faculty of Agriculture, Cukurova University. The seeds of the germplasm-IV have been collected from Irael and Turkey and then grown under same conditions in the greenhouse at the Weizmann Institute of Science. Growth medium used in the pot experiments was a mixture of a tuff, vermiculite and peat that was enriched by the slow release fertilizer Osmocote.

In the studies dealing with the chromosomal localization of the genes affecting high levels of Zn and Fe in seeds 2 different sets of *dicoccoides* substitution lines, e.g., *Triticum aestivum* cv. Chinese Spring (CS)–*Triticum turgidum* ssp. *dicoccoides* and *Triticum turgidum* cv. Langdon (LNG)–*Triticum turgidum* ssp. *dicoccoides*. The development of the Chinese Spring (CS)–*Triticum dicoccoides* substitution lines has been initiated by Dr. E. Sears and completed by Dr. M. Feldman and E. Millet (Weizmann Institute of Science). The seeds of the Chinese Spring (CS)–*Triticum dicoccoides* have been grown under same conditions in the greenhouse at the Weizmann Institute of Science as described above. The Langdon (LNG)–*Triticum dicoccoides* substitution lines have been developed by Joppa and Cantrell (1990), and the seeds of this germplasm were obtained from the Gene Bank of the Kansas State University (Wheat Genetics Resource Center). The seeds were grown under greenhouse conditions at the Wheat Genetics Resource Center, Kansas State University.

Seeds have been analyzed for Zn, Fe, Mg, P, and S by ICP-AES (inductively coupled plasma-atomic emission spectrometry, Jobin Yvon-Paris), and measurements were checked using the certified mineral nutrient values in durum wheat flour samples obtained from the National Institute of Standards and Technology (Gaithersburg, MD). The reference material used was the durum wheat flour (8436).

## RESULTS

### Genetic variability for Zn and Fe among dicoccoides accessions

The Fe and Zn concentrations in seeds of *dicoccoides* accessions from four different sources are given in Table 1. Across all accessions grown in field, Fe concentrations of the seeds varied from 15 to 109 mg kg$^{-1}$ with an average of 46 mg kg$^{-1}$, and Zn concentration varied between 30 to 118 mg kg$^{-1}$ with an average of 61 mg kg$^{-1}$. For Fe concentration, the range observed in seeds

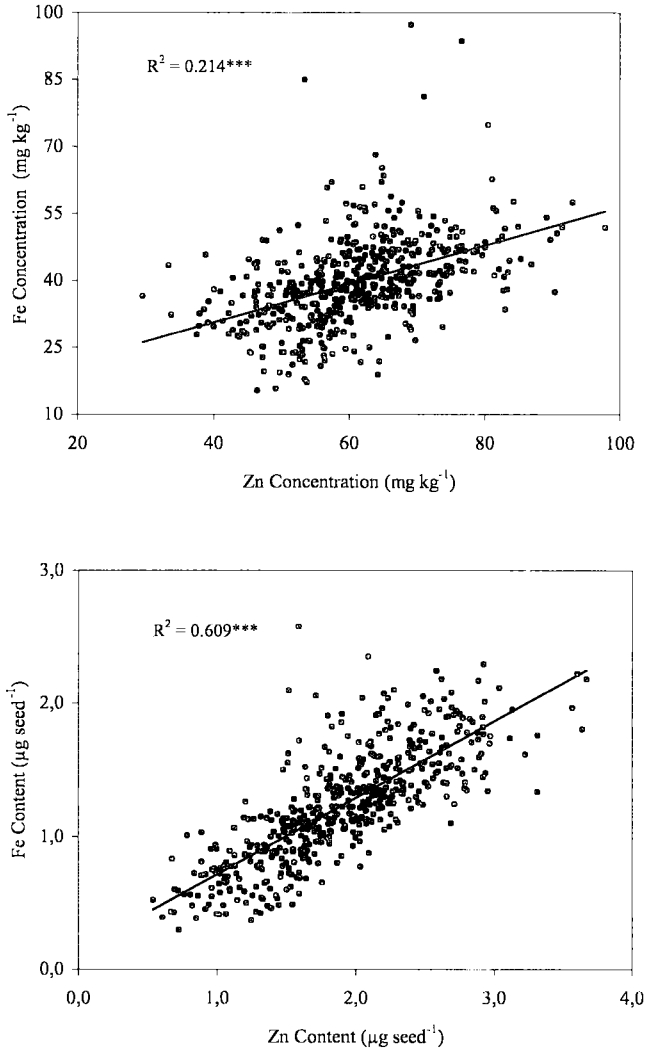


**Fig. 2.** Relationship between seed concentration and content of Zn and Fe in the germplasm-II containing 518 accessions.

from field and greenhouse experiments were very similar. By contrast, the Zn concentrations of seeds obtained from the greenhouse conditions were much larger than found in the field, varying nearly 14-fold and reaching up to 190 mg $kg^{-1}$ (Table 1). Table 1 also provides total amount of Zn per seed (content). The variation for Zn content in seed was much greater when compared to the variation found for the concentration. This indicates large differences for seed weights among accessions. However, the accessions with a very high concentration of Fe (> 80 mg $kg^{-1}$) and Zn (> 150 mg $kg^{-1}$) had also a high seed weight (or seed size), and had, consequently, the highest total amount of Fe (> 2.4 μg Fe per seed) and Zn (> 5 μg Zn per seed). For example, among the 10 accessions having the highest concentrations of Zn (> 150 mg $kg^{-1}$), 7 accessions showed the highest amount of Zn per seed. A similar picture was found for Fe. As shown in Fig. 2, there was a close relationship

**Table 2.** Range of seed concentrations of Fe and Zn in different modern (cultivated) wheat cultivars.

| Number of genotypes | Fe (mg $kg^{-1}$ dry wt.) | Zn (mg $kg^{-1}$ dry wt.) | Reference |
|---|---|---|---|
| 384 | 30–73 | 27–85 | Welch (2001) |
| 25 | 44–54 | 26–32 | Pomeranz and Dikeman (1983) |
| 27 | 35–56 | 26–40 | Peterson et al. (1986) |
| 132 | 29–57 | 25–53 | Graham et al. (1999) |
| 28[a] | 33–46 | 7–10 | I. Cakmak (unpublished) |

Seeds used for analysis came from different individual experiments or locations. [a]Wheat cultivars grown on a very severe Zn-deficient soil (DTPA-Zn: 0.99 mg $kg^{-1}$) under same field conditions in Central Anatolia.

between the concentrations of Zn and Fe in seeds. In the case of total amount of Zn and Fe per seed (content) the positive correlation between seed Zn and Fe was particularly high indicating existence of common genetic factors affecting seed Zn and Fe accumulation in seeds. The Zn and Fe concentrations of the *dicoccoides* accessions shown in Table 1 were compared with the Zn and Fe concentrations of modern wheat cultivars reported in several screening studies. Although the modern and wild wheat germplasms given in Tables 1 and 2, respectively, were not grown under same conditions, it seems that the variation and absolute values among modern wheat cultivars for seed concentrations of Zn and Fe is much smaller than the variation found among wild wheat accessions.

## Chromosomal localization of genes

To investigate the chromosomal localization of genes affecting high levels of Zn and Fe in seeds of *dicoccoides accessions*, two different *dicoccoides* chromosome substitution lines have been used: Langdon (LNG)–*dicoccoides* (DIC) and Chinese Spring (CS)–*dicoccoides* (DIC). Langdon (LNG) and Chinese Spring (CS) are cultivated durum and bead wheats, respectively. There were marked differences in seed concentrations of Zn and Fe between CS–DIC substitution lines (Table 3). Zinc concentration in the recipient parent CS was 20 mg $kg^{-1}$ DW, and ranged from 13 to 62 mg $kg^{-1}$ DW between the substitution lines. The lines having similar Zn concentration to their parent CS were 1A, 7A, 1B, 2B, 3B, and 7B. There was only one line (the 5A chromosome line) showing lower Zn concentration than its parent CS. The DIC chromosomes 2A, 4A, 6A, 5B, and particularly 6B exhibited higher Zn concentrations than CS. The 6B substitution increased the Zn concentration by a factor 3 compared to the recipient parent CS. The substitution lines with the highest Zn concentrations had also the highest amount of Zn per seed. The 5A substitu-

**Table 3.** Seed concentrations of Zn and Fe of Chinese Spring (bread wheat) / *Triticum dicoccoides* substitution lines grown in greenhouse.

| Genotype | Zn | | Fe | |
|---|---|---|---|---|
| | Concentration (mg $kg^{-1}$) | Content (μm $seed^{-1}$) | Concentration (mg $kg^{-1}$ dry wt.) | Content (μm $seed^{-1}$) |
| Chinese Spring (parent) | 20 ± 3 | 0.86 ± 0.11 | 29 ± 1.4 | 1.23 ± 0.06 |
| Substitution lines | | | | |
| 1A | 19 ± 1 | 0.83 ± 0.25 | 35 ± 0.2 | 1.71 ± 0.37 |
| 2A | 37 ± 3 | 1.08 ± 0.09 | 40 ± 4.0 | 1.13 ± 0.20 |
| 4A | 41 ± 1 | 1.45 ± 0.11 | 31 ± 0.6 | 1.11 ± 0.06 |
| 5A | 13 ± 1 | 0.40 ± 0.05 | 24 ± 0.6 | 0.78 ± 0.01 |
| 6A | 50 ± 3 | 1.30 ± 0.17 | 34 ± 1.0 | 0.92 ± 0.03 |
| 7A | 23 ± 2 | 0.75 ± 0.13 | 31 ± 1.4 | 1.03 ± 0.04 |
| 1B | 18 ± 1 | 0.58 ± 0.01 | 24 ± 1.3 | 0.82 ± 0.06 |
| 2B | 20 ± 2 | 0.69 ± 0.03 | 28 ± 0.3 | 0.97 ± 0.08 |
| 3B | 19 ± 7 | 0.86 ± 0.24 | 35 ± 5.6 | 1.21 ± 0.08 |
| 5B | 48 ± 3 | 0.78 ± 0.19 | 51 ± 3.6 | 2.07 ± 0.06 |
| 6B | 62 ± 3 | 1.97 ± 0.21 | 31 ± 1.4 | 1.01 ± 0.07 |
| 7B | 18 ± 1 | 0.59 ± 0.03 | 30 ± 1.3 | 1.00 ± 0.03 |

Each value represents the mean ± SD of 3 replications.

**Table 4.** Seed concentrations of P, Mg, and S of Chinese Spring (bread wheat) / *Triticum dicoccoides* substitution lines grown in greenhouse.

| Genotype | P | Mg | S |
|---|---|---|---|
| | (g $kg^{-1}$ dry wt.) | | |
| Chines Spring (parent) | 3.30 ± 0.03 | 1.06 ± 0.03 | 0.87 ± 0.04 |
| Substitution lines | | | |
| 1A | 2.87 ± 0.16 | 1.17 ± 0.01 | 1.00 ± 0.03 |
| 2A | 4.04 ± 0.04 | 1.33 ± 0.02 | 1.14 ± 0.00 |
| 4A | 4.40 ± 0.04 | 1.47 ± 0.01 | 1.03 ± 0.01 |
| 5A | 2.55 ± 0.08 | 0.99 ± 0.01 | 0.80 ± 0.08 |
| 6A | 4.95 ± 0.01 | 1.62 ± 0.08 | 1.17 ± 0.04 |
| 7A | 3.25 ± 0.04 | 1.32 ± 0.02 | 1.16 ± 0.04 |
| 1B | 3.23 ± 0.01 | 1.18 ± 0.07 | 0.98 ± 0.09 |
| 2B | 2.76 ± 0.37 | 1.14 ± 0.03 | 1.09 ± 0.01 |
| 3B | 3.67 ± 0.01 | 1.49 ± 0.01 | 1.07 ± 0.08 |
| 5B | 4.88 ± 0.33 | 1.69 ± 0.13 | 1.18 ± 0.01 |
| 6B | 4.84 ± 0.10 | 1.45 ± 0.08 | 1.21 ± 0.06 |
| 7B | 2.37 ± 0.19 | 1.17 ± 0.07 | 1.18 ± 0.07 |

Each value represents the mean ± SD of 3 replications.

tion line contained the lowest amount of Zn per seed (Table 3).

Differences in Fe concentration among the DIC chromosome lines were less in comparison to those found for Zn concentration (Table 3). Iron concentration of the parent cultivar CS was 29 mg $kg^{-1}$ DW, and varied between 24 (5A substitution) to 51 (5B line) mg $kg^{-1}$ DW. For Fe concentration, the highest values were found for the 2A substitution with 40 mg $kg^{-1}$ DW and the 5B substitution with 51 mg $kg^{-1}$ DW.

The substitution lines with the highest Zn concentration (6B, 6A, and 5B) had also the highest P concentration in seeds (Table 4). There was a very close relationship between seed Zn and P concentrations, while for Fe this relationship was not observed. In contrast to the concentrations of Zn, Fe, and P, the seed concentration of Mg and S varied very little among the CS–DIC substitution lines (Table 4).

Among the LNG–DIC substitution lines, only the 6B substitution increased the concentrations of Zn and Fe compared to the recipient cultivar LNG (Table 5). Zinc and Fe concentrations of LNG were 41 and 46 mg $kg^{-1}$ DW, respectively, and ranged from 15 to 47 mg $kg^{-1}$ for Zn and 31 to 59 mg $kg^{-1}$ DW for Fe. With exception of the 6B chromosome line nearly all chromosome lines were very similar in their concentrations both for Zn and Fe. For P, Mg, and S concentration very little variation was observed among the LNG–DIC chromosome substitution lines (Table 5).

## DISCUSSION

There is substantial variation in seed concentrations of Fe and Zn among *Triticum dicoccoides* accessions (Table 1). This variation was particularly large for Zn. The accessions with the highest concentrations of Zn and Fe had also the highest total amount (content) of Fe and Zn per seed. These results indicate that the high concentrations of Fe and Zn in seeds were not caused by small seed size, e.g., it is not a consequence of a concentration effect due to small seeds. To our knowledge this study is the first in analyzing such a high number of *dic-*

**Table 5.** Seed concentrations of Zn, Fe, P, Mg, and S Langdon (durum wheat) / *Triticum dicoccoides* substitution lines grown in greenhouse.

| Genotype | Zn | Fe | P | Mg | S |
|---|---|---|---|---|---|
| | (mg kg$^{-1}$ dry wt) | | (g kg$^{-1}$ dry wt) | | |
| LNG | 41 ± 1.53 | 46 ± 3.0 | 4.06 ± 0.02 | 1.30 ± 0.01 | 0.77 ± 0.06 |
| 1A | 26 ± 0.71 | 40 ± 2.1 | 4.24 ± 0.16 | 1.38 ± 0.07 | 0.83 ± 0.07 |
| 2A | 31 ± 2.89 | 39 ± 1.7 | 3.75 ± 0.19 | 1.10 ± 0.11 | 0.67 ± 0.06 |
| 3A | 30 ± 2.08 | 37 ± 1.0 | 3.76 ± 0.02 | 1.09 ± 0.03 | 0.63 ± 0.02 |
| 4A | 21 ± 2.89 | 34 ± 2.1 | 3.77 ± 0.02 | 0.98 ± 0.02 | 0.76 ± 0.02 |
| 5A | 18 ± 2.83 | 39 ± 2.1 | 4.31 ± 0.13 | 1.30 ± 0.02 | 0.98 ± 0.04 |
| 6A | 20 ± 0.71 | 34 ± 2.1 | 3.99 ± 0.03 | 1.23 ± 0.01 | 0.95 ± 0.04 |
| 7A | 24 ± 0.71 | 39 ± 0.0 | 4.13 ± 0.19 | 1.36 ± 0.02 | 0.85 ± 0.03 |
| 1B | 17 ± 1.41 | 35 ± 0.0 | 4.31 ± 0.06 | 1.39 ± 0.06 | 0.89 ± 0.02 |
| 3B | 19 ± 1.41 | 36 ± 2.1 | 3.95 ± 0.18 | 1.13 ± 0.06 | 0.77 ± 0.04 |
| 4B | 15 ± 1.10 | 31 ± 0.6 | 4.16 ± 0.01 | 1.29 ± 0.04 | 0.79 ± 0.01 |
| 5B | 17 ± 0.71 | 35 ± 1.4 | 4.16 ± 0.01 | 1.35 ± 0.01 | 0.80 ± 0.04 |
| 6B | 47 ± 5.51 | 59 ± 6.7 | 4.31 ± 0.02 | 1.29 ± 0.04 | 0.82 ± 0.02 |
| 7B | 17 ± 1.73 | 35 ± 2.3 | 3.78 ± 0.18 | 1.16 ± 0.08 | 0.66 ± 0.08 |

Each value represents the mean ± SD of 3 replications.

*occoides* accessions (825). Using a smaller number of *Triticum dicoccoides* accessions, Cakmak et al. (1999a, 2000) and Ortiz-Monasterio and Graham (2000) have shown that *dicoccoides* accessions contain much higher concentrations of Zn than cultivated wheat. Also in the present study it has been shown that seed concentrations of Zn and Fe in modern cultivated wheats (Table 2) are much lower and less variable than those of wild wheat accessions (Table 1). Seed yield per plant can greatly affect seed concentrations of mineral nutrients by a "dilution effect" when yield is very high (e.g., seed is big and plumb) or by "concentration effect" when yield is very low and the seeds often shriveled (Marschner 1995). There are several examples in literature showing that Zn and Fe concentrations in seed are inversely related to seed yield of plants (Peterson et al. 1986; Feil and Bänziger 1993; Bänziger and Long 2000). Unfortunately, we have no seed yield data for these accessions. Presently, field and greenhouse experiments are in progress to estimate the effect of seed yield of the most promising *dicoccoides* accessions on very high concentrations of Zn and Fe in seeds. As discussed above, high seed Zn and Fe concentrations are also associated with high Zn and Fe content, and the seed concentration of other nutrients (P, Mg, S) did not show such high variation as found for Zn and Fe. These observations suggest that increased seed concentration of Fe and Zn in certain *dicoccoides* accessions is under genetic control and cannot be entirely attributed to differences in seed yield. In a large study at CIMMYT with bread wheat, no negative linkage was found between grain yield potential and concentration of micronutrients in seeds (Graham et al. 1999; Ortiz-Monasterio and Graham 2000). Existence of very significant correlation between seed Zn and Fe concentration (Fig. 2) indicates that the genetic factors affecting Zn and Fe concentration in seeds are co-segregated. There are many reports supporting the positive correlation between seed Fe and Zn (Pomeranz and Dikeman 1983; Peterson et al. 1986; Graham et al. 1999; Rengel et al. 1999). It seems that enhancements in seed Zn concentration can be associated with simultaneous increases in Fe concentration or vice versa.

The 6B chromosome substitution line had both the highest Zn concentration and Zn content (total amount of Zn per seed). Interestingly, the 6B chromosome of *Triticum dicoccoides* is also supposed to carry genes for high protein content in seeds (Joppa et al. 1997; Che et al. 2001). Joppa et al. (1997) developed a mapping population derived from a cross between modern tetraploid durum wheat (cv. Langdon) and *Triticum dicoccoides* and found that gene(s) for high protein concentration (named QGpc.ndsu-6Bb) is (are) located very close to the centromere of 6B. A marker could explain 66% of the phenotypic variation in high protein content of seeds. In several other studies a very high positive correlation between seed protein and seed Zn (and also Fe) was found (Pomeranz and Dikeman 1983; Peterson et al. 1986; Zebarth et al. 1992; Feil and Fossati 1995). In case that the genes determining high levels of Zn and protein are closely linked on chromosome 6B, selection and/or breeding for high Zn concentration in seeds may result in simultaneously high levels of seed protein. This relationship between Zn and protein opens an important research area in the future, and detailed studies are needed to clarify the role of *Triticum dicoccoides* genes in root uptake, shoot transport and seed accumulation of

Zn and also N.

Increased levels of Zn and Fe in the DIC chromosome lines 6B, 6A, and 5B were not accompanied with higher levels of other nutrients, and the variation in concentrations of P, Mg, and S among the DIC chromosome substitution lines was considerably lower than the variation found for Fe and Zn. Only in the case of CS–DIC substitution lines, there was a close relation between P and Zn concentrations in seeds (Tables 3 and 4). These results indicate that increased concentration of Fe and Zn in 6B, 6A, and 5B chromosome lines is not a consequence of differences in seed yield. Similarly, in the studies with the LNG-DIC substitution lines, high protein concentration in seeds of the chromosome line 6B was not related to the seed weight or grain yield (Joppa and Cantrell 1990; Cantrell and Joppa 1991).

Interestingly, though the tetraploid turgidum wheats (e.g., ssp. *dicoccoides*, ssp. *polonicum*, ssp. *dicoccon*) are rich in seed Zn concentrations (Cakmak et al. 1999a, 2000; Ortiz-Monasterio and Graham 2000), they are, however, extremely sensitive to Zn deficient soils (Cakmak et al. 1999a). In most cases, genotypes having higher tolerance to Zn-deficient soils contain similar or even lower concentrations of Zn in shoot or seed compared to genotypes with higher susceptibility to Zn deficient soils (Graham et al. 1992; Cakmak et al. 1997b, 1999a, b; Ekiz et al. 1998). Rye and similarly Triticale are excellent crops tolerating Zn deficient soils, but do not accumulate high levels of Zn in seeds (Cakmak et al. 1997a, b; Ekiz et al. 1998) when grown in Zn deficient soils, respectively. These observations indicate that tolerance to Zn deficiency in soils is not controlled by the same genes which encode for enhanced seed concentration of Zn. Even an inverse relationship might be observed. This needs to be considered in breeding genotypes with enhanced levels of Fe and particular Zn concentration in seeds. Wheat cultivars selected for increased Zn and Fe concentration in the seed should be also evaluated for their ability to grow in Zn and Fe deficient soils.

**Conclusions.** In conclusion, the results presented in this work show that *Triticum turgidum* L. var. *dicoccoides* represents a very promising genetic source for improving Zn and Fe concentrations in seeds of modern wheat cultivars. *Triticum dicoccoides* is also a rich source of genetic diversity for several agronomical and nutritionally valuable traits, especially for amino acids and protein (Nevo et al. 1986; Levy and Feldman 1987; Nevo 2001; Nevo et al. 2002). Higher levels of protein and amino acids in seeds can be also beneficial in improving biological bioavailability of micronutrients in the diet (Cakmak et al. 2002; Welch and Graham 2004).

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